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14. ABSTRACT

The aim of this proposal is to determine the differences in radiobiological response of human prostate tissue to conventional and hypofractionated radiotherapy. Specifically, this proposal will characterize the predominant DNA damage response pathway from human prostatectomy specimens in response to conventional and hypofractionated ionizing radiation. Data generated in the first year of study has shown that normal prostate tissue and prostate cancer can be cultured ex vivo using a dynamic culture system and used to study the radiobiology of human prostate tissue. Normal prostate tissue responds to ionizing radiation with predicted repair foci (gamma-H2AX and Rad51), and a marker for cellular stress, p53. The DNA damage response in normal glands appears to predominate in the basal cell layer.

15. SUBJECT TERMS
Human Prostate Organ Culture, Ionizing radiation, Hypofractionation, DNA damage repair

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INTRODUCTION:

The aim of this prostate cancer training grant is to provide specialized training and research that will enable me to begin a career as a physician-scientist with a focus on prostate cancer. The training component of the grant includes scientific and clinical mentorship, specialized training in prostate cancer radiotherapy, scientific writing, presentation at national meetings, and career development sessions. The focus of the research component is the proposal to determine the differences in radiobiological response of human prostate tissue to conventional and hypofractionated radiotherapy using a unique *in vitro* model system for culturing human prostate tissue obtained from prostatectomy specimens. The primary radiobiological endpoint is to determine the predominant DNA damage response pathway involved in repair of double-strand breaks in both the epithelial and stromal components of human prostate tissue. By studying the DNA damage response of human prostate tissue, unique response signatures can be identified that may serve as biomarkers for clinical outcome after radiotherapy.

BODY:

Research Program:

Aim 1, Task 1:

The initial phase of the research involved learning the technical skills to perform prostate organ culture as well as optimizing the conditions to maintain appropriate viability in the tissue slices. The methodology of prostate organ culture involves obtaining prostate cores from the transitional zone after prostatectomies followed by precision cutting of the cores into 275 to 300 micron thick sections with a Brendel-Vitron tissue slicer. The cores are placed onto titanium roller culture inserts and loaded into glass scintillation vials containing 1.7 ml of medium. The prostate tissue slices are then placed into the dynamic organ culture incubator which is unique from other tissue incubators in that it rotates the scintillation vials, causing the slices to be alternately submerged and then exposed to ambient gases. This allows for better exchange of gases and nutrients. Conditions were optimized and we found that the normal epithelial and cancer components of the tissue slices were maintained for up to five days in culture, however at that point there was much heterogeneity within the slice in terms of viability. Therefore it was determined that the optimal timeframe to conduct the proposed experiments would be within three days of initiating culture. The final media conditions included Medium 199 supplemented with 5% FBS, 1mM dihydrotestosterone, 0.1mM dexamethasone, 2mg/ml insulin, 200mM glutamine, 250mg/ml fungizone, 50mg/ml gentimycin, and 100U/ml penicillin/streptomycin.

As demonstrated in Figure 1 and 2 (see Supporting Data), these conditions allowed cultured normal and malignant prostate epithelium to retain tissue-specific markers such as cytokeratin 5/14 and prostate-specific antigen. In addition, proliferation of normal prostate basal cells was observed while prostate cancer tissues lost the proliferative marker, Ki67, while in culture. Additional study is ongoing to further optimize media conditions to mimic tissue characteristics *in vivo*.

Aim 1, Task 2:

We demonstrated in Aim 1, Task 1 that the optimal timeframe to conduct the experiments involving ionizing radiation would be within three days of initiating culture due to a decrease in tissue viability beyond 3 days. This time limitation in culture altered our approach to comparing the DNA damage response to conventional and hypofractionated radiation. The methodology we used for irradiation experiments includes the following: After 1 day in culture tissue slices were irradiated with a single fraction of 2 or 8 Gy. Slices were fixed in formalin and paraffin-embedded at 1, 8, 24, and 48 hours after radiation treatment. Immunohistochemistry was performed to evaluate for phosphorylation of H2AX on serine 139 (gamma-H2AX), p53, and Rad51 using DAB chromogen.

Figure 3 (see Supporting Data) demonstrates that normal prostate epithelial components display early and late markers of the DNA damage response following irradiation with 8 Gy. The predicted timing of repair proteins were displayed with the early marker of DNA double-strand breaks, gamma-H2AX, responding at 1 hour post-irradiation (Figure 3A) while Rad51, a protein involved in homologous repair, was not induced until 8 hours post-irradiation (Figure 3B). As predicted, the 8 Gy treated tissues had an amplified DNA damage response in comparison to the 2 Gy samples (data not shown). It was also observed that the DNA damage response predominated in the basal cell layer of the epithelium (Figure 3A & B) which is in agreement with others (1). The DNA damage response in prostate cancer tissues is currently being evaluated.

Aim 1, Task 3:

The DNA damage response in prostate cancer specimens is currently being evaluated. Pending collection of intact prostate cancer specimens, the relationship between DNA damage responses to Gleason score will be evaluated.

Aim 1, Task 4:

Each experiment creates a tissue repository for further study which will be available for creation of a microarray.

Aim 2, Task 1:

As outlined in Aim 1, Task 1, the initial phase of the research involved learning the technical skills to perform prostate organ culture as well as optimizing the conditions to maintain appropriate viability in the tissue slices. There are technical difficulties with retaining viability of the stromal tissue in culture. This is can be demonstrated by viewing hemotoxylin and eosin stained specimens after organ culture which display a loss of stromal cell viability throughout the specimen (Figures 1 &2, H&E panels).

This technical problem limits the ability to study the damage response within the stromal tissue following irradiation. We are continuing to optimize the media conditions to retain stromal integrity. Additionally, we are attempting to correspond with a group from Johns Hopkins University School of Medicine that recently published work using prostate organ culture (1). This group may have the expertise to solve our technical problems.

Aim 2, Task 2:

There are technical difficulties with retaining viability of the stromal tissue in culture as outlined in Aim 2, Task 1 which makes this task difficult to perform. We are continuing to work to eliminate this technical problem.

Aim 2, Task 3:

Each experiment creates a tissue repository for further study. Pending elimination of technical issues with retaining stromal tissue, a microarray will be generated.

Aim 3, Tasks 1-3:

Conditioned media from Specific Aims 1 and 2 are collected and frozen at -80 degrees Celsius. Analysis of the conditioned media with a PSA specific ELISA will be performed in the coming year of the funding period.

Training Program:

Focus Area 1: Mentor Guidance.

Dr. Anne E. Cress is my mentor on this grant. During the funding period she and I have weekly meetings to discuss the research project. Our meetings focus on technical assistance, data analysis and future directions of the project. We have met after each scientific and technical conference I have attended during the funding period to discuss relevant abstracts and brainstorm new research avenues based on topics discussed at national meetings. I have been mentored on abstract preparations (see Reportable Outcomes) and given an opportunity to present my data prior to national meetings at the Cress laboratory meeting. In addition, Dr. Cress is the Deputy Dean for Research and Academic Affairs at the College of Medicine. Therefore, with her expertise, she has been able to provide me with specific mentorship regarding career development and planning for research independence.

Given my project is dependent on collection of fresh human prostatectomy specimens which can vary in their availability, Dr. Cress has encouraged translating the core concepts of my research project into cell line based work in order to increase scientific efficiency. This has resulted in additional abstracts submitted during the funding period (see Reportable Outcomes).

Focus Area 2: Clinical Prostate Cancer Training.

This grant is incorporated into my residency training in radiation oncology. Throughout the granting period I am involved in quality assurance rounds weekly. This provides a venue to critically review patient radiotherapy plans. We review both external beam and brachytherapy plans for prostate cancer patients prior to initiating treatment. To this point in the funding period I have received 6 months of weekly didactic sessions in both radiation physics and radiation biology. Both of these topics will be critical to my performance as a practicing clinician and researcher.

Treatment of prostate cancer as a radiation oncologist requires knowledge and expertise of both external beam radiation and brachytherapy. Brachytherapy is a specialized technique in which a physician places radioactive seeds within the prostate. An essential part of the training program is to obtain training in this technique. In September 2010 I attended the Ultrasound-Guided Transperineal Brachytherapy course and in June 2011 I attended the High Dose Rate Brachytherapy course, both were offered through the Seattle Prostate Institute. The programs focused on all aspects of brachytherapy including: patient selection, physics and dosimetry planning, technique, quality assurance, complications, setting up an implant program, post implant dosimetry and patient follow-up. Attending these training programs is a critical step to becoming a prostate cancer expert.

Focus Area 3: Participation in the Prostate Cancer Research Programs at the Arizona Cancer Center.

During the funding period I have been involved with the Prostate Cancer Research Programs. I have attended the Prostate Cancer Metastasis and Signaling Group weekly and was able to present a component of my research in this forum in October of 2010. My talk was entitled: “Blocking Integrin Function Combined with Ionizing Radiation for the Eradication of Bone Metastasis”.

The weekly Prostate Program Core Meeting has also been fundamental to my progress. At this meeting I discuss and review results with my collaborator, Dr. Raymond Nagle. Dr. Nagle is a pathologist with expertise in prostate cancer. I had an opportunity to present my data in this forum prior to attending the IMPaCT conference in March 2011. My talk was entitled “Application of an In Vitro Prostate Organ Culture System to Study the Radiobiology of Intact Human Prostate Tissues”.

Focus Area 4: National Meetings/Seminars.

As a result of the training grant, I have had the privilege to present my data at national meetings. Meetings which I attended and presented an abstract are listed below (see Reportable Outcomes):

1. American Society for Radiation Oncology 52nd Annual Meeting. October 2010
2. Department of Defense Innovative Minds in Prostate Cancer Today (IMPaCT) Conference. March 2011

Focus Area 5: Career Development Roundtables.

Physician-scientists face a difficult challenge to balance both research and patient care. A core component of this training grant was to attend specific mentoring sessions geared for physician-scientists at both local and national meetings. A list of mentoring/networking sessions is provided below:

1. Translational Medicine, San Francisco, July 2010. This meeting provided insight into cutting-edge translational research as well as targeting early-career investigators with mentoring sessions.
   Mentoring Lunch with M. Celeste Simon, PhD, Abramson Cancer Center, University of Pennsylvania.
2. Molecular Biology in Clinical Oncology, an AACR Educational Workshop, July 2010.

This Educational workshop provided hands-on training in research techniques, didactic teaching, and a grant writing workshop.
Mentoring sessions were throughout the program with faculty.

3. Department of Defense Innovative Minds in Prostate Cancer Today (IMPaCT) Conference, March 2011. This meeting had many opportunities for networking. I was able to obtain research and career advice from Dr. Peter Nelson, a principal investigator on the Pacific Northwest Prostate Cancer SPORE.
4. 3B Research Forum: Benchtop to Bedside and Back, Atlanta, May 2011. This forum was specific to radiation oncologists addressing the need for continued basic and translational research. It featured a specific educational session entitled "How to survive as a translational scientist".

KEY RESEARCH ACCOMPLISHMENTS:

Research Program:

- Normal prostate tissue and prostate cancer can be cultured *ex vivo* using a dynamic organ culture system.
- Tissue-specific markers such as cytokeratin 5/14 and prostate specific antigen are retained in culture.
- Proliferation of normal prostate basal cells are observed in culture while the normal stromal component cannot be maintained.
- Normal prostate tissue responds to ionizing radiation with predicted repair foci (gamma-H2AX and Rad51) and a marker for cellular stress, p53.
- The DNA damage response in normal prostate tissues appears to predominate in the basal cell layer.

Training Program:

- Three abstracts presented at national meetings.
- Specialized training in prostate brachytherapy.

REPORTABLE OUTCOMES:

Research Program:

Abstracts:

Sroka TC, Gandolfi AJ, Nagle RB, Lutz W, Sokoloff MH, and Cress AE. Application of a prostate organ culture system to study the radiobiology of intact human prostate tissue. *Department of Defense Innovative Minds in Prostate Cancer Today (IMPaCT) Conference.* 2011

Sroka TC, Pawar SC, Pond GD, Nagle RB, and Cress AE. Blocking integrin function combined with ionizing radiation for eradication of bone metastasis. *American Society for Radiation Oncology 52nd Annual Meeting. 2010*

Sroka TC**,** Cameron R, Nagle RB, and Cress AE. Overcoming cell adhesion mediated radiation resistance by altering A6B1 integrin function. *Radiation Research Society 56th Annual Meeting. 2010*

Training Program:
Ultrasound-guided Transperineal Brachytherapy for Prostate Cancer
Training Course, Seattle Prostate Institute, Swedish Medical Center, Seattle WA.

High Dose-Rate Brachytherapy for Prostate Cancer Training Course, Seattle Prostate Institute, Swedish Medical Center, Seattle WA.
American Association for Cancer Research Workshop, Molecular Biology in Clinical Oncology, The Given Institute, Aspen CO.

American Brachytherapy Society Seattle Prostate Fellowship

CONCLUSION:
Research Program:

The aim of this proposal is to determine the differences in radiobiological response of human prostate tissue to conventional and hypofractionated radiotherapy. Specifically, this proposal will characterize the predominant DNA damage response pathway from human prostatectomy specimens in response to conventional and hypofractionated ionizing radiation. Data generated in the first year of study has shown that normal prostate tissue and prostate cancer can be cultured *ex vivo* using a dynamic culture system and used to study the radiobiology of human prostate tissue. Normal prostate tissue responds to ionizing radiation with predicted repair foci (gamma-H2AX and Rad51), and a marker for cellular stress, p53. The DNA damage response in normal glands appears to predominate in the basal cell layer.

In the next year of study we will determine the cytoarchitecture and kinetics of the DNA damage response to hypofractionated and conventional ionizing radiation in human prostate cancer tissues. We will also further optimize the organ culture conditions to mimic the indolent nature of prostate tissues and to retain the integrity of the prostatic stroma. In addition, this work will create a tissue repository for future study.

Training Program:

My ultimate career goal is to contribute to the field of prostate cancer research and therapy as an accomplished academic physician. The training plan has provided mentor guidance with an established prostate cancer researcher and enabled networking with both clinical and basic science colleagues interested in prostate cancer research. In addition, it has fostered my development as both a clinician and a scientist through specialized training in prostate brachytherapy and by securing a competitive fellowship at a national research workshop for early-career investigators. Therefore, this award has provided specific training for managing both clinical and independent research skills necessary to develop a career in academic medicine.

“So What”:

Clinically relevant research is fundamental for advances in cancer therapies. The research plan being investigated has direct application to problems in prostate

cancer radiotherapy currently being investigated in clinical trials. The research and training plan will enhance my technical and analytical skills in basic research and provide specialized training to succeed as a clinician. Therefore, this training grant will provide a foundation to pursue a career with a focus on translational prostate cancer research.

REFERENCES:

1) Jaamaa S, Hallstrom T, Sankila A, Rantanen V, Koistinen H, Stenman U, Zhang Z, Yang Z, De Marzo A, Taari K, Ruutu M, Andersson L, and Laiho M. DNA Damage Recognition via Activated ATM and p53 Pathway in Nonproliferating Human Prostate Tissue. *Cancer Research* 70(21) Nov 1, 2010.

APPENDICES:

None

SUPPORTING DATA:

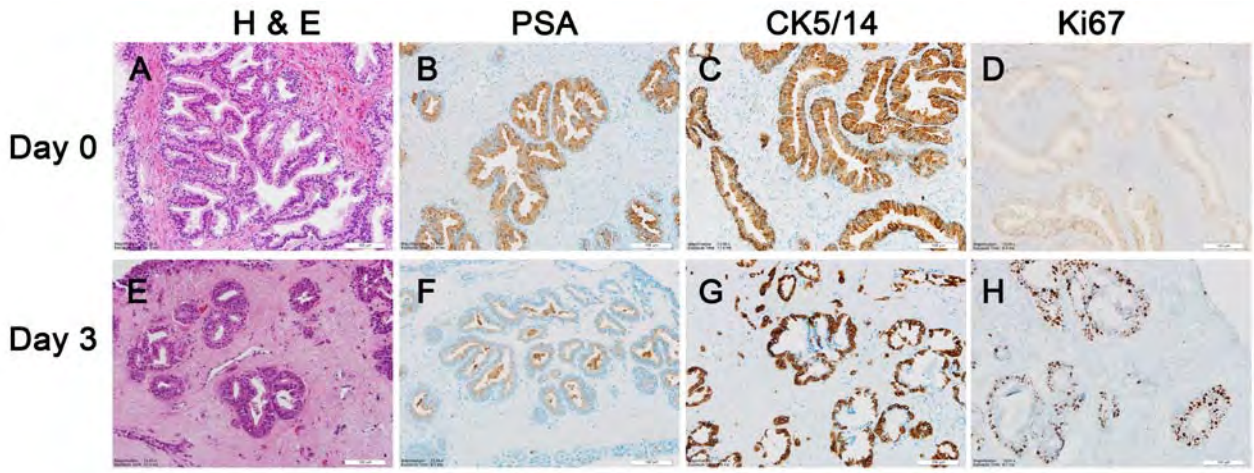

Figure 1. Cultured normal prostate tissues retain tissue-specific markers and demonstrate basal cell proliferation. Precision-cut slices were generated from the transitional zones of human prostate specimens . They were cultured in Medium 199 supplemented with 5% FBS, 1mM dihydrotestosterone, 0.1mM dexamethasone, 2mg/ml insulin, 200mM glutamine, 250mg/ml fungizone, 50mg/ml gentimycin, and 100U/ml penicillin/streptomycin. Slices were cutured for 3 days. Slices were fixed in formalin and paraffin-embedded. Slices were stained with hemotoxylin and eosin (A &E) and immunohistochemistry was performed to evaluate for PSA (B&F), cytokeratin 5/14 (C&G) and Ki67 (D&H) using DAB chromogen.

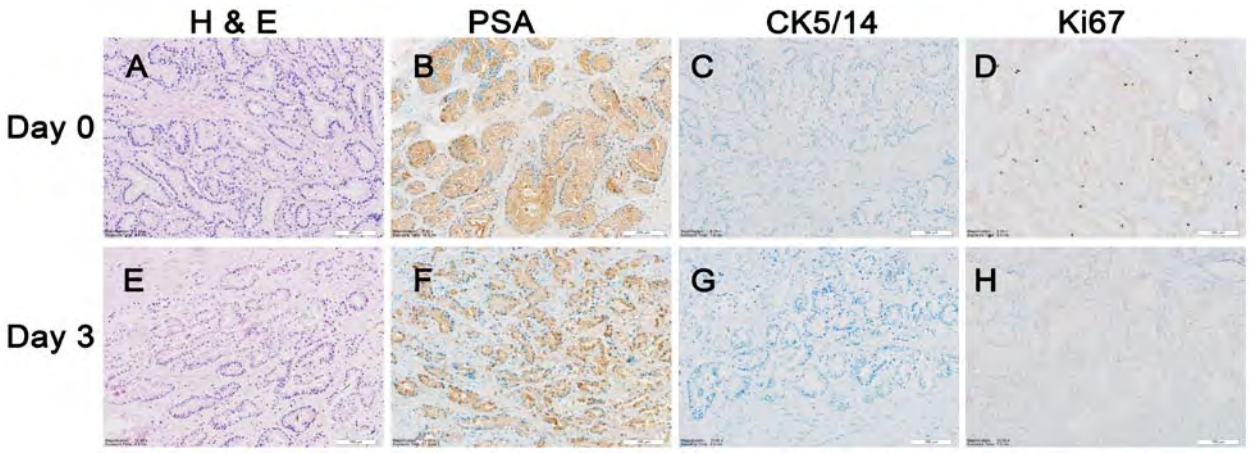

Figure 2. Cultured prostate cancer tissues retain tissue-specific markers and cease proliferation. Tissues were cultured as described in Figure 1. Slices were stained with hemotoxylin and eosin (A &E) and immunohistochemistry was performed to evaluate for PSA (B&F), cytokeratin 5/14 (C&G) and Ki67 (D&H).

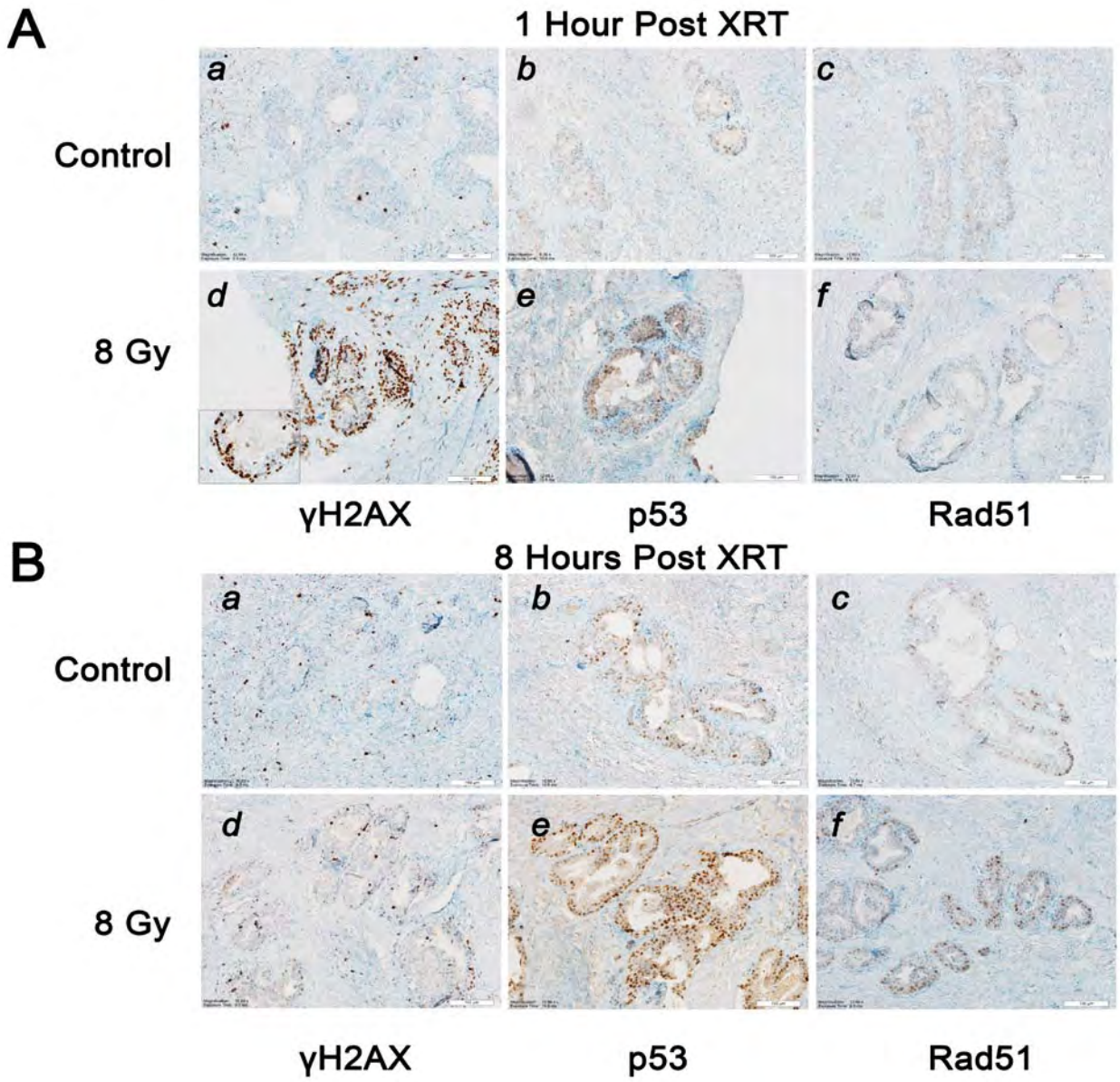

Figure 3. Cultured normal prostate tissues demonstrate early and late markers of the DNA damage response following treatment with ionizing radiation. Tissues were cultured as described in Figure 1. After 1 day in culture they were irradiated with a single fraction of 8 Gy. Slices were fixed in formalin and paraffin-embedded 1 hour (A) or 8 hours (B) after radiation treatment. Immunohistochemistry was performed to evaluate for phosphorylation of H2AX on serine 139 (γH2AX, a&d), p53 (b&e), and Rad51 (c&f) using DAB chromogen.